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Exhibit B

Shulman May 9

THE CONSTRUCTION OF CHIMERIC MOUSE/HUMAN ANTIBODIES FOR USE IN CANCER THERAPY

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ABSTRACT

The use of human monoclonal antibodies of predefined specificity in human therapy has been limited by the lack of antigen-specific human lymphoid cells. An alternative approach has made use of murine monoclonal antibodies where immunization of the mice permits us to generate antibody producing cells of virtually any specificity. However, these antibodies have been found to provoke a deleterious immune response in humans. By using available recombinant DNA techniques I have devised a system whereby chimeric immunoglobulin genes composed of human constant regions and mouse variable regions will be transferred and expressed in mutant hybridoma cells. The unlimited combinations of variable and constant region genes which can be integrated in such a system will make possible the production of chimeric monoclonal antibodies of any defined specificity.

The production of mouse "hybridomas" (1) capable of secreting monoclonal antibodies against specific antigenic determinants has had enormous impact on nearly all areas of the life sciences. Briefly, specific hybridoma cell lines are derived by fusing myeloma tumor cells with specific antibody producing spleen cells from an immunized animal. From the myeloma tumor cell parent comes the capacity for unlimite growth in culture and from the spleen cell, the capacity for synthesizing the specifi antibody.

It was recognized early on that the administration of specific antibodies might provide precise, safe and convenient therapy. In particular, several experimental systems indicated that using tumor specific antibodies as specific toxins could be effective in causing tumor regression (2-6). The use of hybridoma antibodies for this purpose is in its infancy. However, a number of groups have approached this problem by immunizing mice with tumors to generate tumor specific hybridomas. One case in particular, where murine antibodies specific for the surface immunoglobulin of a B-lymphoma were used to eliminate the tumor, indicates that this approach can work (7). On the other hand, the administration of specific mouse antibodies would be expected to elicit an immune response in humans against the mouse antibodies themselves, and in fact this has been found to occur in patients receiving renal allografts (8), thereby, severely limiting the duration of the anti-T cell treatment. While it is not yet established whether the immune response is directed against the variable or constant regions of the murine antibodies, many groups have begun to generate human monoclonal antibodies in the hope of circumventing this problem.

To date, efforts to establish general methods of producing human monoclonal antibodies of specific antigenicity have had limited success. The first human monoclonal antibodies used mouse or rat myeloma lines for fusion (9-11). These hybrids produced large amounts of immunoglobulin but were inherently unstable and preferentially lost their human chromosomes and thus lost their ability to produce antibodies. More recently, several groups have reported that fusions with various human myelomas can yield stable hybridomas (12-13). Another approach has used Epstein-Barr Virus (EBV) (14) to transform human B cells into permanent antibody producing cell lines (15-16). This method however may be hazardous as EBV might still be present in antibody preparations.

Nevertheless, it seems likely that some variation on these methods will ultimately provide cell lines secreting human immunoglobulin. Whatever the outcome, these methods suffer from the severe restriction that humans cannot in general be immunized. For this reason, we cannot expect humans to provide the antibody specificities which will be required.

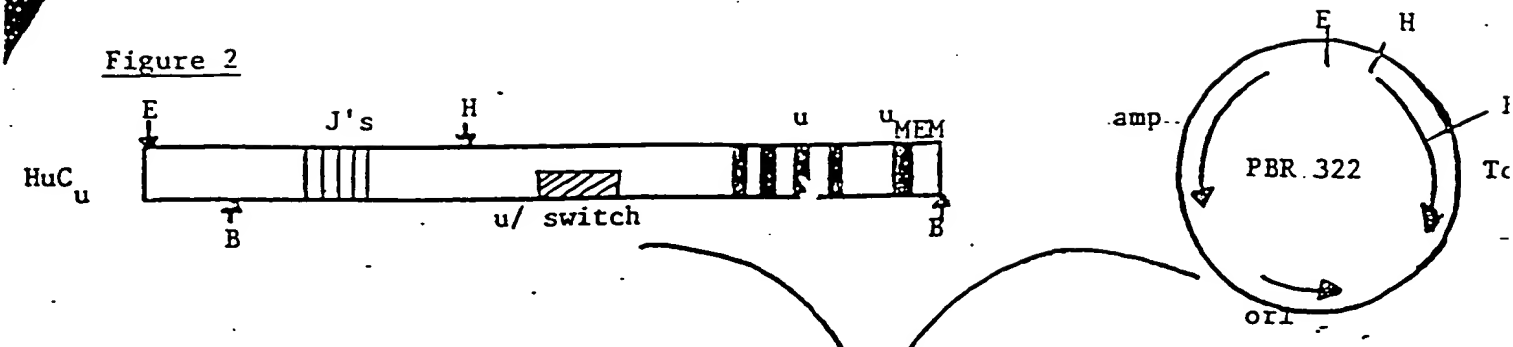
The objective of my proposal is to examine a possible method for overcoming the limited antigenic specificity of the human hybridoma techniques as well as the problem that murine monoclonal antibodies are immunogenic in humans. I describe below a method for making chimeric antibodies in which the variable regions, derived from an immunized mouse, are joined to human constant regions. In this way, I hope to generate specific antibodies which themselves would not elicit a destructive immune response in humans.

The basic approach consists of constructing a plasmid vector containing a chimeric gene in which the DNA segments for the appropriate mouse variable and human constant regions are joined. These plasmid vectors will then be transferred to special hybridoma cells which are expected to express the chimeric genes as functional antibody. A particularly convenient system worked out in our lab by Ochi, et al (see attached pre-print, 17), can be adapted for this purpose. This system derives from the hybridoma SP6 which secretes IgM(k) specific for the hapten trinitrophenyl (TNP) (18). The DNA segment encoding the TNP specific k (k_{TNP}) was cloned in the vector pSV2-neo (19), which carries the bacterial gene neo which can be expressed in animal cells and render them resistant to the antibiotic G-418, an analogue of neomycin. In order to transfer the k_{TNP} gene, bacteria bearing the k_{TNP}-neo^r plasmid were fused with mutant hybridoma cells which lack the k_{TNP} gene but still express the u heavy chain. Cells resistant to G-418 were selected and expression of the k_{TNP} gene was then detected easily as TNP specific IgM(k). A similar system which expresses the cloned u_{TNP} DNA in a $k^+u^-_{TNP}$ hybridoma is currently being analyzed in depth (A.Ochi, personal communication). Furthermore, a system has been developed to transfer both light and heavy chain genes, contained on the same vector, and functional IgM antibody is produced (A.Ochi, personal communication). The application of this technology to assay the expression of our chimeric genes will also require that the appropriate transcriptional and translationa signals are present and recognized by the mouse cell. There is, however, preliminary evidence indicating that mouse immunoglobulin genes are expressed when transferred into human pre-B cells (N. Hozumi, personal communication) thereby suggesting that such heterologous constructs may be recognized and expressed in various cell lines.

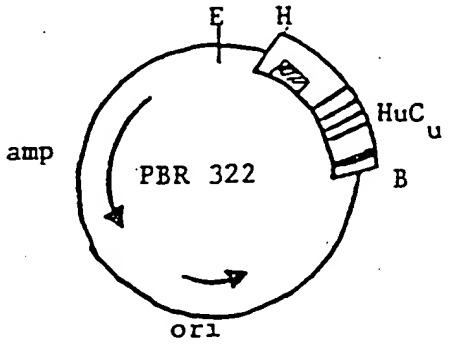
The scheme for constructing the chimeric k and u genes is described in figures 1 and 2. The rearranged k_{TNP} gene has been cloned and thus serves as a source of k_{VTNP} (donated by R.G. Hawley, 20). Similarly, the cloned gene encoding the TNP specific u chain has been made available to us (G.Kohler, manuscript in preparation) as has the human C_k and C_u regions (P. Leder, 21-23). To date, the chimeric mouse/human TNP specific immunoglobulin k light chain gene has been subcloned in pSV2-neo and will be transferred via protoplast fusion (24) to a $k^- u^+_{TNP}$ hybridoma cell line. I will then test whether expression of the chimeric k_{TNP} light chain restores functional IgM production. Expression of the TNP specific heavy chain genes will also be assayed similarly using $u^- k^+$ hybridoma mutants as described above for the murine u_{TNP}.

My aim in this project has been to take advantage of the available technology in our laboratory to assay for the expression of functional antibody by chimeric mouse/human immunoglobulin genes. In this way, I can test whether variable region genes can be transposed to various constant region genes while maintaining functiona activity. In this proposal I have described a system to test the specific transposition of mouse k_{TNP} and u_{TNP} variable regions to human C_k and C_u. If these constructions produce functional antibody, this technique could then be applied to generate chimeric antibodies of any antigenic specificity. It will then be possible to test whether this method provides a general source of specific antibody useful in therapy.

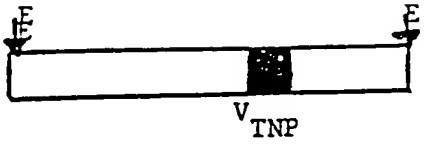
Figure 2



Bam Hl + HindIII cleavage
Ligation of HuC$_u$ constant region into PBR^{u}322 B/H site

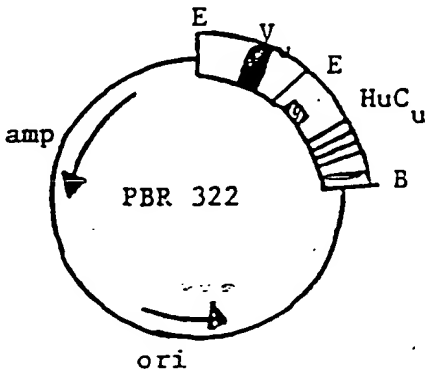


Mouse V$_{uTNP}$



Isolation of Rl fragment

Hind III + Eco Rl cleavage
Filling in ends; Blunt end ligation of Rl linkers;
Ligation of V region into PBR 322 Eco Rl site.



Bam Hl + partial Eco Rl cleavage;
Isolation of B/E fragment (u)

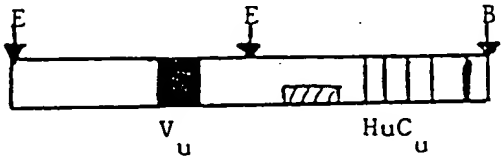


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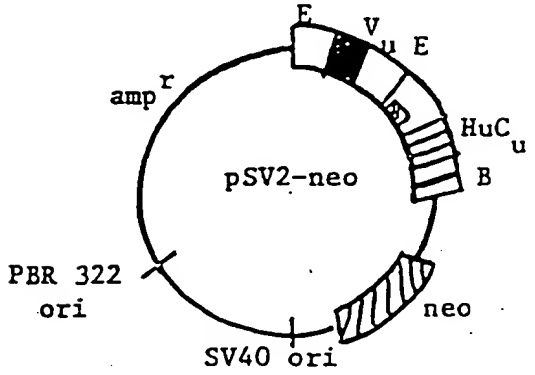
Figure 2 Con't

Insertion into B/E site of pSV2-neo;
Selection for amp^r, neo^r.



Conversion of bacteria to protoplasts and fusion to k^+u^- hybridoma cell.

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